

Claims

1. A method of nucleic acid amplification, comprising the steps of:

- A. providing a plurality of primers that are immobilised but that have one end exposed to allow primer extension;
- B. allowing a single stranded target nucleic acid molecule to anneal to one of said plurality of primers over part of the length of said single stranded nucleic acid molecule and then extending that primer using the annealed single stranded nucleic acid molecule as a template, so as to provide an extended immobilised nucleic acid strand;
- C. separating the target nucleic molecule from the extended immobilised nucleic acid strand;
- D. allowing the extended immobilised nucleic acid strand to anneal to one of said plurality of primers referred to in step A) and then extending that primer using the extended immobilised nucleic acid strand as a template, so as to provide another extended immobilised nucleic acid strand; and optionally,
- E. separating the annealed extended immobilised nucleic acid strands from one another.

2. A method according to claim 1, further comprising:

- F. using at least one extended immobilised nucleic acid strand to repeat steps D) and E), so as to provide additional extended immobilised nucleic acid strands and, optionally,
- G. repeating step F) one or more times.

3. A method according to claim 1 or claim, wherein said single-stranded target nucleic acid is produced by providing a given nucleic acid sequence to be amplified (which sequence may be known or unknown) and adding thereto a first nucleic acid sequence and a second nucleic acid sequence; wherein said first nucleic acid sequence hybridises to one of said plurality of primers and said second nucleic acid sequence is complementary to a sequence which hybridises to one of said plurality of primers.

claim 1

4. A method according to any preceding claim; wherein said single-stranded target nucleic acid is produced by providing a given nucleic acid sequence to be amplified (which sequence may be known or unknown) and adding thereto a first nucleic acid sequence and a second nucleic acid sequence; wherein said first nucleic acid sequence hybridises to one of said plurality of primers and said second nucleic acid sequence is the same as the sequence of one of said plurality of primers.

SUB B

5. A method according to claim 3 or claim 4 wherein said first and second nucleic acid sequences are provided at first and second ends of said single-stranded target nucleic acid.

SUB E3

6. A method according to any of claims 3 to 5, wherein a tag is also added to the given nucleic acid sequence, said tag enabling amplification products of the given nucleic acid sequence to be identified.

SUB 4

7. A method according to any preceding claim wherein the plurality of primers is a plurality of primers that have the same sequence.

8. A method according to any of claims 1 to 6, wherein the plurality of primers comprises at least two different types of primer, one type having a different sequence from another type.

9. A method according to claim 8, wherein the plurality of primers consists of 2^n different types of primer; wherein n is an integer.

10. A method according to claim 9, where n is 2.

SUB D

11. A method according to any of claims 8 to 10, wherein the different types of primer are present in substantially the same concentrations as one another.

SUB B

12. A method according to any preceding claim, wherein the primers are substantially homogeneously dispersed over a given area

claim 1

13. A method according to any preceding claim, wherein the primers are located in a predetermined arrangement (e.g. in a grid pattern)

Sub B1

14. A method according to any preceding claim, wherein a supply of nucleotides and a nucleic acid polymerase are used to extend primers

Sub B2

15. A method according to any preceding claim, wherein heating is used to separate annealed nucleic acid strands

a

16. A method according to claim 15 when dependent upon claim 14, wherein the nucleic acid polymerase is not rendered inactive by the heating conditions used to separate annealed nucleic acid strands.

Sub B3

17. A method according to claim 16, wherein said nucleic acid polymerase is taq polymerase, is another polymerase that is derivable from a thermophilic organism; or is a thermostable derivative thereof.

18. A method according to any preceding claim, wherein said primer extension results in the incorporation of one or more detectable labels (e.g. fluorescent labels or radiolabels) into extended immobilised nucleic acid strands.

19. A method according to any preceding claim, further including the step of treating one or more extended immobilised nucleic acid strands so as to release a nucleic acid molecule or a part thereof.

20. A method according to claim 19, wherein said treating consists of cleavage with a restriction endonuclease or with a ribozyme.

a

21. A method according to any preceding claim, wherein one or more of said primers has a restriction endonuclease recognition site or a ribozyme recognition site or has part of such a site, which part becomes complete when primer extension occurs.

a

22. A method according to any preceding claim that is automated to allow repeated cycles of nucleic acid amplification.

claim 1

23. A method according to any preceding claim, when used to amplify a plurality of different nucleic acid sequences

Sub E

24. A method according to claim 23, when used to amplify a plurality of different nucleic acid sequences simultaneously.

25. A method according to claim 23 or 24, wherein said different nucleic acid sequences are each provided with a first and second nucleic acid sequence as described in any of claims 3 to 5, said first and (second) nucleic acid sequences being the same for the each of the different nucleic acid sequences.

claim 23

26. A method according to any of claims 23 to 28, wherein said different nucleic acid sequences are each provided with a different tag so that the different sequences can be distinguished from one another.

27. A plurality of immobilised nucleic acids producable by a method according to any preceding claim.

28. A plurality of immobilised nucleic acids in the form of one or more distinct areas on a surface, each area comprising a plurality of identical nucleic acid strands and a plurality of identical complementary strands thereto; wherein each nucleic acid strand within such an area is located so that another nucleic acid strand is located on the surface within a distance of the length of that strand.

Sub B

29. A plurality of immobilised nucleic acids according to claim 27 or claim 28, wherein there is at least one distinct area present per mm^2 of surface on which the nucleic acids are immobilised.

30. A plurality of immobilised nucleic acids according to claim 28, wherein the number of distinct areas/ mm^2 of surface on which the nucleic acids are immobilised is greater than 1, greater than 10^2 , greater than 10^3 or greater than 10^4 .

31. The use of a method according to any of claims 1 to 26 or of a

plurality of immobilised nucleic acid molecules according to any of claims 27 to 30 in providing nucleic acid molecules for sequencing.

32. The use according to claim 31, wherein sequencing is performed by extending primers hybridised to single stranded nucleic acid molecules and detecting the nucleotides used in primer extension.

33. The use according to claim 32, wherein sequencing is performed in a step-by-step manner, whereby after extending a primer by a single nucleotide, the nucleotide is detected before a further nucleotide is used in primer extension.

34. The use according to claim 32 or 33, wherein said primers hybridise to the same sequences as the primers used in the method of claim 1 to provide the immobilised nucleic acid molecules for sequencing.

35. The use according to claim 32 or 33, wherein said primers have the same sequences as the primers used in the method of claim 1 to provide the immobilised nucleic acid molecules for sequencing.

36. The use according to claim 31, wherein nicks are provided in double-stranded nucleic acid molecules, nick translation is performed and the nucleotides used in nick translation are detected.

37. The use according to claim 31, wherein an RNA polymerase is used to synthesise an RNA strand from a DNA molecule and the nucleotides used to produce said RNA strand are detected.

38. The use according to any of claims 32 to 37, wherein all or at least some of said nucleotides are labelled nucleotides.

39. The use according to claim 38, wherein said labelled nucleotides all have the same label.

40. The use according to claim 38 or claim 39, wherein said labelled nucleotides are fluorescence labelled

41. The use according to any of claims 38 to 40, wherein a mixture of labelled and non-labelled nucleotides are used.

42. The use according to claim 41, wherein the labelled nucleotides comprise less than 50 % of the nucleotides used.

43. The use according to claim 42, wherein the labelled nucleotides comprise less than 10 % of the nucleotides used.

44. The use according to any of claims 31 to 43, wherein the sequencing is parallel sequencing of nucleic acid molecules present in at least 2 different distinct areas.

45. The use according to any of claims 31 to 43, wherein the sequencing is parallel sequencing of nucleic acid molecules present in over 10 different distinct areas.

46. The use according to any of claims 31 to 43, wherein the sequencing is parallel sequencing of nucleic acid molecules present in over 100 different distinct areas.

47. The use according to any of claims 31 to 43, wherein the sequencing is parallel sequencing of nucleic acid molecules present in over 1000 different distinct areas.

48. The use according to any of claims 31 to 43, wherein the sequencing is parallel sequencing of nucleic acid molecules present in over 1000000 different distinct areas.

49. The use according to any of claims 31 to 43, wherein a plurality of different sequences are determined in parallel.

50. The use of a method according to any of claims 1 to 26 or of a plurality of immobilised nucleic acid molecules according to any of claims 27 to 30 in providing amplified nucleic acid molecules for diagnosis.

51. The use of a method according to any of claims 1 to 26 or of a plurality of immobilised nucleic acid molecules according to any of claims 27 to 30 in providing amplified nucleic acid molecules for screening.

52. The use of a method according to any of claims 1 to 26 or of a plurality of immobilised nucleic acid molecules according to any of claims 27 to 30 in providing amplified nucleic acid molecules to be used as a support for other components.

53. The use of a method according to any of claims 1 to 26 or of a plurality of immobilised nucleic acid molecules according to any of claims 27 to 30 in generating additional nucleic acid molecules in free (rather than immobilised) form.

54. The use of a method according to claim 53 in *in situ* RNA synthesis

55. The use of a method according to any of claims 1 to 26 or of a plurality of immobilised nucleic acid molecules according to any of claims 27 to 30 in monitoring gene expression

56. The use of a method according to any of claims 1 to 26 or of a plurality of immobilised nucleic acid molecules according to any of claims 27 to 30 in identifying nucleic acid molecules with gene products that are rarely expressed.

57. The use of a method according to any of claims 1 to 26 or of a plurality of immobilised nucleic acid molecules according to any of claims 27 to 30 in identifying heterozygous individuals

58. The use of a method according to any of claims 1 to 26 or of a plurality of immobilised nucleic acid molecules according to any of claims 27 to 30 in nucleic acid fingerprinting

59. An apparatus for performing a method as described in any of claims 1 to 26; comprising a plurality of immobilised primers, a

160260 160260 160260 160260 160260

a
a
B

claim

nucleic acid polymerase, a plurality of nucleotides and means for separating annealed nucleic acid strands.

60. An apparatus according to claim 59, wherein the means for separating annealed nucleic acid strands comprises a controlled heating means.

61. An apparatus for analysing a plurality of nucleic acid molecules according to any of claims 21 to 30, wherein said apparatus comprises a source of reactants and detector means for detecting one or more signals produced after said reactants have been applied to said nucleic acid molecules.

62. An apparatus according to claim 61 wherein said detector means has sufficient resolution to distinguish between the distinct areas referred to in claim 28.

63. An apparatus according to claim 61 or claim 62 comprising a charge coupled device (CCD).

64. An apparatus according to claim 63 wherein said charge coupled device (CCD) is operatively connected with a magnifying device (e.g. a microscope).

65. A kit for use in screening, diagnosis or in nucleic acid sequencing; comprising a plurality of immobilised nucleic acid according to any of claims 21 to 30.

66. The invention substantially as hereinbefore described.

Adm E8